PB80-181175

Teratology of a Zineb Formulation

Midwest Research Inst.
Kansas City, MO

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

Feb 80

U.S. Department of Commerce
National Technical Information Service
NTIS.

PB80-181175

United States
Environmental Protection
Agency

Health Effects Research
Laboratory
Research Triangle Park NC 27711

EPA-600/1-80-017
February 1980

Research and Development

EPA

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TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/1-80-017		PB80 181175

4. TITLE AND SUBTITLE	5. REPORT DATE
Teratology of a Zineb Formulation	February 1980
	6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Robert D. Short, Jan L. Minor, Timothy M. Unger, Bradley Breeden, Dan VanGoethem and Cheng-Chun Lee	

9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Midwest Research Institute 425 Volker Boulevard Kansas City, Missouri 64110	1EA615
	11. CONTRACT/GRANT NO.
	68-02-2982

12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Health Effects Research Laboratory RTP,NC U.S. Environmental Protection Agency Office of Research and Development Research Triangle Park, North Carolina 27711	14. SPONSORING AGENCY CODE EPA 600/11

15. SUPPLEMENTARY NOTES

16. ABSTRACT

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17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Toxicology Teratology Fungicide	Zineb formulation Rats Mice	06F,T

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to public	UNCLASSIFIED	
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE

EPA Form 2220-1 (9-73)

EPA-600/1-80-017
February 1980

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by

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Contract No. 68-02-2982

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

"Because the fungicide zineb is applied to the foliage of a variety of fruits and vegetables, it is important to determine the hazard to humans associated with its use. The present study evaluates the teratogenic potential of one zineb formation in both rats and mice."

F. G. Hueter, Ph.D.
Director
Health Effects Research Laboratory

ABSTRACT

The purpose of the present study was to evaluate the teratogenic potential of a zineb formulation. An initial toxicity study indicated that oral doses of 1,000 or 2,000 mg/kg/day adversely affected the weight gain of nonpregnant rats but not nonpregnant mice. In the teratology study, pregnant rats and mice received daily oral doses of 0, 200, 632, or 2,000 mg/kg from day 6 of gestation until the day before C-section. Maternal welfare, as monitored by body weight and food consumption, was affected only in rats that received 2,000 mg/kg/day of the formulation. Evidence of embryo or fetal lethality was not present in rats or mice. However, fetuses from rats that received 2,000 mg/kg/day of the formulation had a reduced body weight. Some anomalies were significantly increased in rats that received 2,000 mg/kg/day of the formulation. These anomalies included hydrocephalus, split centra, incompletely ossified frontal bones, and enlarged occipital fontanel. None of the anomalies observed in mice were increased to a statistically significant level in any of the groups treated with the formulation. This study indicated that the zineb formulation produced anomalies in rats at doses which adversely affected maternal welfare. In addition, there was no evidence of teratogenicity in mice treated with similar doses.

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I. INTRODUCTION

Zineb (zinc ethylenebisdithiocarbamate) is a protective fungicide used for foliage application on a variety of fruits and vegetables. Since this fungicide may contaminate some foods consumed by humans, it is imperative to understand the hazards associated with its use. Accordingly, the purpose of the present study was to evaluate the teratogenic potential of a zineb formulation in both rats and mice.

The present study was divided into two phases. During the first phase, toxicity data were obtained for virgin rats and mice. This information was used to identify doses for the second phase. During this phase, pregnant rats and mice were treated with the formulation during organogenesis and their offspring were examined for birth defects.

II. METHODS

A. Animals

CD rats and CD-1 mice were obtained from the Charles River Breeding Laboratory (Wilmington, Massachusetts) and housed in our animal quarters for at least 7 days prior to use. These quarters are maintained at 22 $\pm$ 4°C with a relative humidity of 40 to 60% and a 7 AM to 7 PM photoperiod. Animals were given free access to rodent chow (Wayne Lab-Blox, Allied Mills, Inc., Chicago, Illinois) and tap water.

B. Dose

1. Source and analysis: A zineb fomulation (Dithane Z-78, Lot No. 0170) was used in this study. The formulation was received from Rohm and Haas (Philadelphia, Pennsylvania) on November 15, 1978. The formulation was repackaged in glass jars on December 6, 1978, wrapped in aluminum foil and stored in the refrigerator. After repackaging samples of the formulation were sent to Dr. Don Gowers (Research Division, Rohm and Haas, Spring House, Pennsylvania) for analysis. In addition, at the completion of both the toxicology and teratology studies, additional samples of the formulation were sent to Dr. Gowers on March 20, 1979, for analysis.

2. Preparation: Corn oil was selected as the vehicle for dosing since zineb was reported to be more stable in a nonaqueous medium. In order to prepare the suspension, the formulation was added to a weighed amount of cold pressed corn oil which was reported to be free of

preservatives (Hain Pure Food Compnay, Los Angeles, California) and mixed for 5 to 10 sec with the aid of a polytron (Brinkmann Instruments, Westbury, New York). Suspensions were prepared every 2 to 3 days and stored at refrigerator temperature in glass bottles wrapped with aluminum foil. The actual weight of corn oil and zineb formulation used to prepare 50 ml of the various dosing solutions is shown below. For the calculation of these values it was determined that 0.5 g of zineb formulation occupies a volume equivalent to 1 ml of corn oil and that the density of our corn oil was 0.92 g/ml. All doses are expressed in terms of the formulation.

	Weight (g) of	
Dose (mg/kg)	Zineb Formulation	Corn Oil
200	2.00	45.1
250	2.50	44.8
500	5.00	43.7
632	6.32	43.1
1,000	10.00	41.4
2,000	20.00	36.8

3. Administration: All doses, which are expressed in terms of the zineb formulation, were administered by oral intubation in a volume of 5 ml/kg.

C. Toxicity Study

Five groups each consisting of 10 virgin female CD rats and 10 virgin female CD-1 mice received 0, 250, 500, 1,000, and 2,000 mg/kg/day of the zineb formulation. The treatment period was 14 days for rats and 11 days for mice. Body weights were determined at the start and end of treatment, and food consumption was measured during treatment.

D. Teratology Study

1. Mating: Sexually mature virgin female CD rats and CD-1 mice were housed overnight with a proven male breeder. In the morning rats were examined for sperm-positive vaginal smears and mice were observed for vaginal copulating plugs. The morning evidence of mating was obtained and identified as day 0 of gestation.

2. Treatment: Four groups, each consisting of at least 60 mated rats and mice, received by oral intubation 0, 200, 632, or 2,000 mg/kg/day of the zineb formulation. The treatments were administered in daily doses from day 6 of gestation until the day before C-section.

3. Maternal observations: Dams were observed for toxicological responses. Their body weight was determined at the beginning and end of treatment and at the time of C-section. In addition, food consumption was determined during and after treatment.

4. Fetal observations: Pregnant rats and mice were sacrificed on gestational days 20 and 18, respectively. A laparotomy was performed and the uterine horns exposed. The number and position of live, dead, and resorbed fetuses were recorded. Live fetuses were removed, weighed and immediately examined for external anomalies as described by Wilson.[1]

One-half of the viable fetuses from each litter were dissected and examined for soft tissue anomalies by the free-hand slicing method of Wilson.[1] Each fetus was fixed in 20 to 25 ml of Bouins fluid for 2 weeks. The hardened fetuses were examined for external anomalies and serially cut from the head through the trunk using a sharp razor blade. No slices were made beyond the kidneys and the intestines were carefully removed from the pelvic cavity. The cross-sections of the fetuses and the genitourinary organs on the pelvic floor were carefully examined by experienced personnel. The remaining viable fetuses from each litter were processed for skeletal examination. Fetuses were fixed in 70% alcohol for 2 weeks and enviscerated. The fetuses were stored in 1% KOH for 2 days and then stained with alizarin red.[2] After differential decolorization, the skeletons were examined by experienced personnel for anomalies.

E. Interpretation of Data

1. Statistics: Quantitative data are reported as the mean $\pm$ standard error. These data were analyzed by Bartlett's test for homogeneity.[3] Homogeneous data were analyzed by Dunnett's procedure or Tukey's omega procedure.[3] Heterogeneous data were analyzed by a nonparametric rank test.[4] The level of statistical significance was selected as $p < 0.05$ unless indicated otherwise. The litter was considered the experimental unit. The percentage of fetuses with a given anomaly was calculated for each litter, and these values were averaged to provide a measure of the affected fetuses per litter.

2. Ranking of anomalies: The various anomalies have been assigned a rank. The ranking system is based on our subjective feeling as to the value of a particular anomaly in predicting the teratogenic potential of a compound. Accordingly, anomalies with a rank of 1 have little value in such predictions while anomalies with a rank of 4 are more valuable. The rank is indicated by each anomaly in the various tables. In addition, the various groups of anomalies are summarized by rank at the end of each table.

III. RESULTS

A. Analysis of Zineb Formulation

The chemical analyses of the zineb formulation (Dithane Z-78, Lot 0170) used in this study is presented in Table 1. From these data, Dr. Gowers concluded in a letter to Dr. Ron Baron (Environmental Protection Agency, Research Triangle Park, North Carolina) dated May 2, 1979, that there was no evidence of progressive degradation of the product, or of build-up of ETU. The overall averages for all the determinations were 85.5% EBDC $\pm$ S.D. 0.8% (n = 12) and 0.35% ETU $\pm$ S.D. 0.16% (n = 10).

B. Toxicity Study

Five groups each consisting of 10 rats and 10 mice received daily doses of 0, 250, 500, 1,000 or 2,000 mg/kg of the zineb formulation. The treatment period lasted for 14 days in rats and 11 days in mice. No deaths occurred in either rats or mice which were attributed to the formulation. Two deaths occurred in rats as the result of esophageal perforation during dosing. The body weight and food consumption of rats and mice are presented in Table 2. The daily weight gain was reduced in rats that received 1,000 or 2,000 mg/kg/day of the formulation. The weight change of mice was not significantly affected at any of the doses studied. In addition, food consumption was not reduced at any of the doses studied.

C. Teratology Study in Rats and Mice Treated With a Zineb Formulation

1. Maternal welfare and reproduction

a. Rats: None of the parameters used to monitor maternal welfare and reproduction were significantly affected in rats treated with 200 or 632 mg/kg/day of a zineb formulation (Table 3). In contrast, rats treated with 2,000 mg/kg/day of the formulation had a reduced body weight and consumed less feed than did the controls. In addition, fetuses from these rats weighed less than from the controls.

b. Mice: None of the parameters used to monitor maternal welfare and reproduction were significantly affected in mice treated with 200, 632, or 2,000 mg/kg/day of a zineb formulation (Table 4).

TABLE 1

ANALYSIS OF DITHANE Z-78 LOT 0.170

Bag	Original		MRI Before Study		MRI After Study	
	EBDC %	ETU %	EBDC %	ETU %	EBDC %	ETU %
A	84.5	0.29	86.4	0.40	84.9	0.34
	85.0	0.27	86.3	-	85.2	0.34
B	84.5	0.27	86.3	0.80	85.2	0.28
	84.9	0.28	86.9	-	85.5	0.27

TABLE 2

BODY WEIGHT AND FOOD CONSUMPTION OF RATS AND MICE TREATED WITH A ZINEB FORMULATION

Dose[a] (mg/kg/day)	Body Weight (g) Initial	Final	g/day	Food Consumption (g/animal/day)
Rats				
0	214 ± 3[b]	242 ± 3	1.99	15.8 ± 0.5
250	210 ± 4	232 ± 6	1.56	14.8 ± 0.4
500	209 ± 3	234 ± 6	1.80	16.4 ± 0.6
1,000	210 ± 4	226 ± 5	1.18[c]	14.8 ± 0.5
2,000	211 ± 4	227 ± 6	1.17[c]	14.3 ± 1.0
Mice				
0	23.8 ± 0.4	23.9 ± 0.5	0.01	3.8 ± 0.1
250	23.4 ± 0.4	24.4 ± 0.4	0.09	4.1 ± 0.2
500	24.7 ± 0.5	25.0 ± 0.4	0.03	3.8 ± 0.1
1,000	23.9 ± 0.4	23.3 ± 0.4	-0.05	3.6 ± 0.1
2,000	24.1 ± 0.4	24.5 ± 0.2	0.04	4.3 ± 0.1[c]

a/ Dose of zineb formulation administered to virgin rats and mice for 14 and 11 days, respectively.

b/ Mean ± SE or mean.

c/ Significantly different from control (Dunnett's test).

TABLE 3

EFFECT OF A ZINEB FORMULATION ADMINISTERED DURING ORGANOGENESIS ON MATERNAL WELFARE AND REPRODUCTION IN RATS

	Zineb Formulation (mg/kg/day)			
	0	200	632	2,000
Number Treated	26	27	26	26
Pregnant	25	26	22	24
Alive	25	26	22	24
Non-pregnant	1	1	4	2
Alive	1	1	3	2
Body Weight (g/rat)				
Day 0	225 ± 3	225 ± 3	228 ± 3	226 ± 4
6	250 ± 3	249 ± 4	251 ± 3	248 ± 4
9	259 ± 3	258 ± 4	264 ± 6	245 ± 3
12	273 ± 4	271 ± 4	270 ± 4	258 ± 4[a/]
15	285 ± 4	282 ± 5	283 ± 5	267 ± 4[a/]
20	340 ± 6	337 ± 6	331 ± 8	318 ± 7
Food Consumption (g/rat/day)				
Days 6-9	12.4 ± 2.1	10.9 ± 1.8	11.0 ± 1.7	6.9 ± 1.3[b/]
9-12	20.1 ± 1.4	20.3 ± 0.6	17.6 ± 1.1	16.2 ± 0.7[b/]
12-15	17.9 ± 1.1	19.7 ± 0.6	18.1 ± 0.6	16.0 ± 0.7[b/]
15-20	21.7 ± 0.5	22.2 ± 0.5	20.8 ± 0.6	18.3 ± 0.7[b/]
Pregnant Survivors	25	26	22	24
Implants/Dam	12.9 ± 0.6	12.5 ± 0.5	12.5 ± 0.7	13.0 ± 0.6
Viable fetuses (%)	85 ± 4	76 ± 6	80 ± 7	85 ± 6
Dead fetuses (%)	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Early resorptions (%)	9 ± 3	9 ± 3	4 ± 2	9 ± 4
Late resorptions (%)	6 ± 3	15 ± 5	16 ± 7	5 ± 4
Dams with complete resorptions	0	2	2	2
Live Liters	25	24	20	22
Fetuses/Dam	11.0 ± 0.8	10.2 ± 0.7	11.3 ± 0.9	12.4 ± 0.5
Males (%)	58 ± 4	46 ± 4	55 ± 5	48 ± 2
Fetal weight (g)	3.91 ± 0.07	3.84 ± 0.07	3.81 ± 0.06	3.58 ± 0.08[a/]

a/ Significantly different from control (Dunnett's procedure).
b/ Significantly different from control (two-sample rank test).

TABLE 4

EFFECT OF A ZINEB FORMULATION ADMINISTERED DURING ORGANOGENESIS ON MATERNAL WELFARE AND REPRODUCTION IN MICE

	Zineb Formulation (mg/kg/day)			
	0	200	632	2,000
Number Treated	28	28	28	28
Pregnant	26	23	26	24
Alive	26	23	26	22
Non-pregnant	2	5	2	4
Alive	2	5	2	3
Body Weight (g/mouse)				
Day 0	28.4 ± 0.4	28.5 ± 0.4	28.5 ± 0.4	28.4 ± 0.4
6	30.8 ± 0.3	30.6 ± 0.4	31.0 ± 0.5	30.5 ± 0.4
9	32.6 ± 0.3	32.4 ± 0.4	32.5 ± 0.5	32.5 ± 0.4
12	37.3 ± 0.4	36.9 ± 0.5	37.3 ± 0.7	36.6 ± 0.5
15	43.6 ± 0.5	43.5 ± 0.7	43.5 ± 0.8	42.8 ± 0.7
18	51.6 ± 0.6	52.0 ± 1.2	51.7 ± 1.0	50.9 ± 1.0
Food Consumption (g/mouse/day)				
Days 6-9	4.9 ± 0.2	4.9 ± 0.1	4.9 ± 0.2	4.4 ± 0.4
9-12	5.3 ± 0.2	5.2 ± 0.1	5.3 ± 0.1	5.2 ± 0.1
12-15	5.7 ± 0.2	5.9 ± 0.2	5.5 ± 0.2	5.4 ± 0.2
15-18	5.9 ± 0.2	6.3 ± 0.2	6.4 ± 0.2	6.4 ± 0.1
Pregnant Survivors	26	22	26	22
Implant/Dam	12.3 ± 0.4	11.6 ± 0.5	11.9 ± 0.4	11.6 ± 0.4
Viable fetuses (%)	88 ± 4	93 ± 2	92 ± 2	91 ± 2
Dead fetuses (%)	0 ± 0	1 ± 1	0 ± 0	0 ± 0
Early resorptions (%)	6 ± 1	5 ± 2	6 ± 2	6 ± 2
Late resorptions (%)	3 ± 1	1 ± 1	2 ± 1	2 ± 1
Dams with complete resorptions	0	0	0	0
Live Litters	26	22	26	22
Fetuses/Dam	10.8 ± 0.6	10.9 ± 0.5	10.9 ± 0.5	10.6 ± 0.5
Males (%)	75 ± 17	45 ± 4	46 ± 3	43 ± 3
Fetal weight (g)	1.25 ± 0.02	1.30 ± 0.03	1.28 ± 0.02	1.29 ± 0.03

2. Gross anomalies

a. Rats (Table 5): External hematomas were observed in all dose levels, including the control group. Short tail and kinked tail were found in the 632 and 2,000 mg/kg/day dose levels. Raised craniums were found in the 200 and 2,000 mg/kg/day levels. One runt was found in one litter in the 632 mg/kg/day dose. In addition, the 200 mg/kg/day dose level had single incidences of mottled skin, upturned snout, and edematous fetus, along with two incidences of short neck and short limbs.

b. Mice (Table 6): External hematomas and kinked tails were found in all dose levels except the control group. Cleft lip and small fetuses were observed in the 200 and 632 mg/kg/day dose groups. Single incidences of abnormally placed eye bulges occurred in the 632 and 2,000 mg/kg/day dose groups. Also, there were single incidences of immature skin and malformed rear legs in the 632 mg/kg/day group and mottled skin in the 200 mg/kg/day group. The only external anomalies observed in the control group were two cases of exencephaly in one litter.

3. Soft tissue anomalies

a. Rats (Table 7): The occurrence of distended esophagus was significantly reduced in both the 200 and 2,000 mg/kg/day dose levels. Also, the incidence of lateral hydrocephalus in the 2,000 mg/kg/day group was significantly increased relative to the other three groups. The summary of anomalies by rank was significantly increased in the group treated with 2,000 mg/kg/day of the formulation.

b. Mice (Table 8): Zineb did not significantly increase, in a dose-related fashion, any of the soft tissue anomalies observed in mice or the summaries by rank.

4. Skeletal anomalies

a. Rats (Table 9): Significant increases were found in the 2,000 mg/kg/day dose level in the incidence of enlarged frontal fontanel, enlarged occipital fontanel and split centra. When the anomalies were summarized by rank this group had a significant increase in the incidence of anomalies with a rank of 1 to 4.

b. Mice (Table 10): Zineb did not significantly increase, in a dose-related fashion, any of the soft tissue anomalies observed in mice.

TABLE 5

GROSS ANOMALIES IN RATS TREATED ON GESTATIONAL DAYS 6-19 WITH ZINEB

		Zineb (mg/kg/day)			
		0	200	632	2,000
Number of					
Litters Affected/Examined	(%)	5/25 (20)	4/24 (17)	4/20 (20)	9/22 (41)
Fetuses Affected/Examined	(%)	6/274 (2)	6/246 (2)	4/226 (2)	55/272 (20)
Gross Anomalies (Rank)[a]					
Hematoma	(2)	1.9 (5)[b]	2.0 (3)	0.8 (2)	1.3 (3)
Mottled Skin	(2)	0 (0)	1.4 (1)	0 (0)	0 (0)
Raised Cranium	(3)	0 (0)	0.8 (1)	0 (0)	2.8 (3)
Snout Upturned	(3)	0 (0)	0.3 (1)	0 (0)	0 (0)
Short Neck	(3)	0 (0)	0.8 (1)	0 (0)	0 (0)
Short Limbs	(2)	0 (0)	0.8 (1)	0 (0)	0 (0)
Short Tail	(3)	0 (0)	0 (0)	0.4 (1)	10.9 (5)
Tail Kinked	(3)	0 (0)	0 (0)	0.4 (1)	16.2 (6)
Runt, Small or Dwarf	(1)	0 (0)	0 (0)	0.8 (1)	0 (0)
Rotund Fetus	(1)	0 (0)	0.8 (1)	0 (0)	0 (0)
Edematous Fetus	(2)	0 (0)	0.3 (1)	0 (0)	0 (0)
Summary by Rank 1-4		1.9 (5)	3.1 (4)	2.0 (4)	18.5 (9)
2-4		1.9 (5)	3.4 (4)	1.2 (3)	18.5 (9)
3-4		0 (0)	1.1 (2)	0 (0)	17.2 (7)[c]
4		0 (0)	0 (0)	0 (0)	0 (0)

a/ Ranked by increasing value in predicting teratogenic potential.

b/ Mean of the percent of fetuses with the indicated anomaly calculated on a litter basis. The number in parentheses is the number of affected litters.

c/ Significant from control (two-sample rank test).

TABLE 6

GROSS ANOMALIES IN MICE TREATED ON GESTATIONAL DAYS 6-17 WITH ZINEB

		Zineb (mg/kg/day)			
		0	200	632	2,000
Number of					
Litters Affected/Examined (%)		1/26 (4)	6/23 (26)	5/26 (19)	3/22 (14)
Fetuses Affected/Examined (%)		2/290 (1)	7/251 (3)	11/283 (4)	3/233 (1)
Gross Anomalies (Rank)[a]					
Immature Skin	(1)	0 (0)[b]	0 (0)	0.3 (1)	0 (0)
Hematoma	(2)	0 (0)	0.4 (1)	0.3 (1)	0.9 (2)
Mottled Skin	(2)	0 (0)	0.4 (1)	0 (0)	0 (0)
Exencephalocele	(4)	0.6 (1)	0 (0)	0 (0)	0 (0)
Eye Bulges Abnormally Placed or Misshaped	(3)	0 (0)	0 (0)	0.3 (1)	0.4 (1)
Cleft Palate, Lip or Face	(4)	0 (0)	0.3 (1)	0.6 (2)	0 (0)
Forepaws Malformed	(4)	0 (0)	0 (0)	0.3 (1)	0 (0)
Tail Kinked	(3)	0 (0)	1.2 (2)	1.6 (1)	0.4 (1)
Runt, Small or Dwarf	(1)	0 (0)	0.8 (2)	0.3 (1)	0 (0)
Summary by Rank 1-4		0.6 (1)	2.7 (6)	3.1 (5)	1.3 (3)
2-4		0.6 (1)	1.9 (4)	2.9 (4)	1.3 (3)
3-4		0.6 (1)	1.5 (3)	2.9 (4)	0.8 (2)
4		0.6 (1)	0.3 (1)	2.5 (4)	0 (0)

a/ Ranked by increasing value in predicting teratogenic potential.

b/ Mean of the percent of fetuses with the indicated anomaly calculated on a litter basis. The number in parentheses is the number of affected litters.

TABLE 7

SOFT TISSUE ANOMALIES IN RATS TREATED ON GESTATIONAL DAYS 6-19 WITH ZINEB

		Zineb (mg/kg/day)			
		0	200	632	2,000
Number of					
Litters Affected/Examined (%)		14/25 (56)	15/24 (62)	10/20 (50)	18/22 (82)
Fetuses Affected/Examined (%)		31/132 (97)	24/118 (20)	18/110 (16)	75/133 (56)
Soft Tissue Anomalies (Rank)[a]					
Hydrocephalus Lateral	(4)	0 (0)[b]	0 (0)	0 (0)	26.3 (12)[c]
Slight	(1)	0.8 (1)	0 (0)	0.8 (1)	18.3 (12)[c]
Hydrocephalus 3rd Ventricle	(4)	0 (0)	0 (0)	0 (0)	3.9 (4)
Slight	(1)	0 (0)	0 (0)	0 (0)	1.3 (2)
Hydrocephalus 4th Ventricle Slight	(1)	0 (0)	0 (0)	0 (0)	1.3 (1)
Trachea Occluded	(1)	0 (0)	2.4 (2)	0 (0)	0.6 (1)
Distended Esophagus	(1)	7.6 (8)	0.7 (1)[c]	2.3 (3)	0 (0)[c]
Agenesis of Lungs	(4)	0 (0)	0.6 (1)	0 (0)	0 (0)
Aortic Valve Medially Displaced	(4)	0 (0)	0 (0)	0 (0)	0.6 (1)
Heart Malformed	(4)	0 (0)	0.6 (1)	0 (0)	0 (0)
Ectopic Heart	(4)	0 (0)	1.0 (1)	0 (0)	0.6 (1)
Hemorrhage in Liver	(3)	0.6 (1)	0 (1)	0 (0)	0.6 (1)
Hemorrhage in Abdomen	(2)	0 (0)	0 (0)	0.7 (1)	0 (0)
Ectopic Stomach	(3)	1.8 (2)	0 (0)	0 (0)	0 (0)
Hydronephrosis	(3)	0.8 (1)	1.4 (1)	0 (0)	2.4 (3)
Slight Enlargement of Kidney Pelvis	(1)	0.6 (1)	0.6 (1)	1.4 (1)	3.9 (2)
Pelvis of Kidney Collapsed	(3)	0 (0)	2.8 (3)	1.7 (2)	0.8 (1)
Ectopic Kidney	(3)	0.6 (1)	1.4 (1)	0.8 (1)	1.8 (2)
Small Kidney	(3)	0 (0)	0 (0)	0 (0)	3.2 (2)
Kidney Pelvis Asymetrical	(2)	0 (0)	0 (0)	0 (0)	0.9 (1)
Urinary Bladder Absent	(4)	0 (0)	0 (0)	0 (0)	0.6 (1)
Distended	(2)	4.7 (4)	0 (0)	1.5 (1)	0 (0)
Hemorrhage of Ductus Venosis	(3)	0 (0)	0.6 (1)	0 (0)	0 (0)
Cryptorchid Testicle	(4)	0 (0)	0 (0)	0 (0)	0.9 (1)
Malplaced Testicle	(2)	0 (0)	0.7 (1)	0 (0)	2.3 (3)
Short Neck	(3)	0 (0)	0.8 (1)	0 (0)	0 (0)
Summary by Rank 1-4		22.9 (14)	21.3 (14)	15.7 (10)	54.3 (18)[c]
2-4		8.9 (7)	6.2 (6)	4.8 (4)	34.6 (15)[c]
3-4		5.1 (6)	3.6 (5)	2.5 (3)	32.6 (15)[c]
4		0 (0)	1.6 (2)	0 (0)	27.7 (14)[c]

a/ Ranked by increasing value in predicting teratogenic potential.

b/ Mean of the percent of fetuses with the indicated anomaly calculated on a litter basis. The number in parentheses is the number of affected litters.

c/ Significant from control (two-sample rank test).

TABLE 8

SOFT TISSUE ANOMALIES IN MICE TREATED ON GESTATIONAL DAYS 6-17 WITH ZINEB

		Zineb (mg/kg/day)			
Number of		0	200	632	2,000
Litters Affected/Examined (%)		13/26 (50)	13/23 (56)	7/26 (27)	9/22 (41)
Fetuses Affected/Examined (%)		21/139 (15)	17/119 (14)	16/136 (12)	13/112 (12)
Soft Tissue Anomalies (Rank)[a]					
Hydrocephalus Lateral Slight	(1)	0.8 (1)[b]	0 (0)	0 (0)	0 (0)
Hydrocephalus 4th Ventricle Slight	(1)	0 (0)	0 (0)	0 (0)	0.8 (1)
Exencephaly	(4)	1.5 (1)	0 (0)	0 (0)	0 (0)
Blood in Tissue by Nasal Passage	(3)	0 (0)	0 (0)	0 (0)	0.8 (1)
Micropthalmia	(4)	0.8 (1)	0 (0)	0 (0)	0 (0)
Eye Intraorbital Hemorrhage	(3)	0.8 (1)	0 (0)	0 (0)	0 (0)
Cleft Palate	(4)	0 (0)	0.6 (1)	1.1 (2)	0 (0)
Trachea Occluded	(1)	6.5 (7)	5.4 (5)	3.3 (3)	3.8 (4)
Short Neck	(3)	0 (0)	0 (0)	0.5 (1)	0 (0)
Enlarged Bronchi	(1)	0 (0)	1.4 (1)	0 (0)	0 (0)
Hemorrhage in Pericardium	(3)	0.5 (1)	0 (0)	0.5 (1)	0 (0)
Small Heart	(2)	0.5 (1)	0 (0)	0 (0)	0 (0)
Stomach Distended	(1)	1.6 (1)	3.6 (4)	0 (0)	2.6 (2)
Hemorrhage in Abdomen	(2)	0 (0)	0.7 (1)	0.5 (1)	0 (0)
Duodenum Enlarged	(3)	0 (0)	0.9 (1)	0 (0)	0.9 (1)
Small Kidney	(3)	0 (0)	0.6 (1)	0.5 (1)	0 (0)
Urinary Bladder Distended	(2)	4.2 (6)	1.7 (2)	3.8 (2)	3.4 (3)
Summary by Rank 1-4		13.8 (13)	13.4 (13)	9.4 (7)	10.5 (9)
2-4		6.3 (7)	3.1 (4)	6.6 (5)	5.0 (5)
3-4		2.6 (2)	1.5 (2)	2.2 (4)	1.7 (2)
4		1.5 (1)	0.6 (1)	1.1 (2)	0 (0)

a/ Ranked by increasing value in predicting teratogenic potential.

b/ Mean of the percent of fetuses with the indicated anomaly calculated on a litter basis. The number in parentheses is the number of affected litters.

TABLE 9

SKELETAL ANOMALIES IN RATS TREATED ON GESTATIONAL DAYS 6-19 WITH ZINEB

Number of		Zineb (mg/kg/day) 0	200	632	2,000
Litters Affected/Examined (%)		23/25 (92)	23/24 (96)	19/19 (100)	22/22 (100)
Fetuses Affected/Examined (%)		103/142 (72)	83/128 (65)	78/116 (67)	128/137 (93)
Skeletal Anomalies (Rank)[a/]					
Basioccipital Inc. Ossified	(2)	0 (0)[b/]	1.0 (1)	0 (0)	6.9 (3)
Squamosal Split	(1)	0 (0)	0.6 (1)	0.8 (1)	0.5 (1)
Squamosal Inc. Ossified	(2)	0.9 (1)	0 (0)	0 (0)	0 (0)
Jugal Inc. Ossified	(1)	0.4 (1)	0 (0)	0.8 (1)	0 (0)
Hyoid Bone Unossified	(1)	4.8 (2)	3.8 (4)	4.2 (4)	4.3 (4)
Inc. Ossified	(1)	6.6 (5)	4.6 (3)	0.7 (1)	2.7 (1)
Nasal Bones Inc. Ossified	(1)	0.4 (1)	0 (0)	0 (0)	0 (0)
Frontal Bones Inc. Ossified	(1)	0.4 (1)	0 (0)	0 (0)	0 (0)
Frontal Fontanel Enlarged	(2)	0 (0)	11.6 (5)	2.0 (1)	34.4 (12)[c/]
Occipital Fontanel Enlarged	(2)	0 (0)	2.0 (2)	0 (0)	17.4 (8)[c/]
Parietals Inc. Ossified	(1)	3.7 (2)	0.6 (1)	0 (0)	0.6 (1)
Interparietals Inc. Ossified	(1)	12.1 (5)	5.2 (4)	2.1 (1)	11.5 (7)
Curved Medially	3	0 (0)	0 (0)	0.8 (1)	0.8 (1)
Supraoccipital Inc. Ossified	(1)	5.5 (3)	7.1 (4)	1.5 (2)	20.8 (10)
Unossified	(2)	0 (0)	0 (0)	0 (0)	0.6 (1)
Extra Ribs	(2)	2.3 (2)	1.9 (3)	1.3 (1)	3.0 (2)
Ribs Inc. Ossified	(3)	0 (0)	0.6 (1)	0 (0)	0.8 (1)
Ribs Fused	(4)	0 (0)	0 (0)	0 (0)	0.9 (1)
Centra Ossified Normally		79.3 (24)	72.4 (23)	77.8 (19)	31.9 (17)
Lobed	(2)	17.4 (11)	22.3 (15)	18.5 (12)	42.3 (21)
Split	(2)	3.3 (4)	5.9 (6)	7.0 (4)	39.6 (20)[c/]
Unossified	(1)	0 (0)	0 (0)	0 (0)	0.9 (1)
Sternebrae Ossified Normally		40.2 (18)	47.5 (22)	54.9 (17)	28.0 (7)
Unossified	(1)	31.0 (15)	26.2 (14)	21.4 (11)	43.0 (17)
Inc. Ossified	(1)	28.3 (17)	25.2 (16)	23.6 (11)	23.0 (17)
Malalignment of Fusion	(3)	2.3 (4)	2.1 (3)	8.9 (6)	9.6 (8)
Lobed	(2)	6.0 (4)	3.0 (3)	2.2 (2)	11.3 (10)
Ischium Unossified	(3)	0.4 (1)	0 (0)	0 (0)	0 (0)
Pubis Unossified	(3)	2.4 (2)	0 (0)	0 (0)	0 (0)
Inc. Ossified	(2)	2.1 (2)	0 (0)	0 (0)	1.6 (2)
Paws Inc. Ossified	(1)	2.0 (1)	0 (0)	0 (0)	0 (0)
Phalanges of Paws Unossified	(1)	0 (0)	0 (0)	0 (0)	0 (0)
Femur Broken and Dislocated		0.6 (1)	0 (0)	0 (0)	0 (0)
Summary by Rank	1-4	70.5 (23)	65.2 (23)	65.5 (19)	94.3 (22)[c/]
	2-4	28.6 (14)	35.5 (18)	34.4 (15)	81.9 (21)
	3-4	2.9 (2)	0.6 (1)	0.8 (1)	5.8 (7)
	4	0 (0)	0 (0)	0 (0)	0.9 (1)

a/ Ranked by increasing value in predicting teratogenic potential.

b/ Mean of the percent of fetuses with the indicated anomaly calculated on a litter basis. The number in parentheses is the number of affected litters.

c/ Significant from control (two-sample rank test).

TABLE 10

SKELETAL ANOMALIES IN MICE TREATED ON GESTATIONAL DAYS 6-17 WITH ZINEB

		Zineb (mg/kg/day)			
		0	200	632	2,000
Number of					
Litters Affected/Examined (%)		21/26 (81)	20/23 (87)	22/26 (85)	14/22 (64)
Fetuses Affected/Examined (%)		53/151 (35)	53/132 (40)	63/147 (43)	26/121 (21)
Skeletal Anomalies (Rank)[a]					
Short Ribs	(2)	0.6 (1)[b]	0 (0)	0 (0)	0 (0)
Extra Ribs	(2)	5.5 (4)	6.4 (6)	8.9 (5)	6.9 (6)
Centra Ossified Normally		99.4 (26)	100.0 (23)	100.0 (26)	100.0 (22)
Sternebrae Ossified Normally		75.0 (26)	68.8 (23)	66.2 (22)	84.4 (22)
Unossified	(1)	0.6 (1)	0 (0)	0 (0)	0 (0)
Inc. Ossified	(1)	3.8 (5)	1.6 (2)	0.5 (1)	3.0 (2)
Lobed	(2)	19.5 (13)	17.7 (11)	25.6 (12)	7.4 (5)
Malalignment of Fusion	(3)	8.8 (7)	16.5 (12)	7.6 (7)	5.1 (6)
Summary by Rank 1-4		36.2 (21)	39.6 (20)	42.1 (22)	21.0 (14)
2-4		33.0 (20)	38.0 (19)	41.5 (21)	18.8 (13)

a/ Ranked by increasing value in predicting teratogenic potential.

b/ Mean of the percent of fetuses with the indicated anomaly calculated on a litter basis. The number in parentheses is the number of affected litters.

IV. DISCUSSION

Adult virgin rats were more sensitive to the zineb formulation than were virgin mice (Table 2). For example, the daily weight gain was reduced in rats that received 1,000 or 2,000 mg/kg/day of the formulation. In contrast, there was no significant effect on weight gain in mice that received up to 2,000 mg/kg/day of the formulation. Mortality was not observed in either rats or mice that received up to 2,000 mg/kg/day of the formulation.

Likewise, pregnant rats were more sensitive to the zineb formulation than were pregnant mice (Tables 3 and 4). Maternal welfare, as monitored by body weight and food consumption, was significantly affected in rats but not mice that received 2,000 mg/kg/day. No adverse effects on maternal welfare were observed in rats that received 200 or 632 mg/kg/day of the formulation or in mice at any of the doses tested.

Embryo or fetal lethality, as monitored by the status of the implants, was not significantly affected in rats or mice at any of the doses tested (Tables 3 and 4). Embryo or fetal toxicity, as monitored by fetal weight, occurred only in rats that received 2,000 mg/kg/day of the formulation.

None of the gross anomalies observed in rats or mice were increased to a statistically significant level at any of the doses tested (Tables 5 and 6). However, fetuses from rats that received 2,000 mg/kg/day of the formulation appeared to have a higher incidence of short tails and kinky tails. In addition, when the anomalies were summarized by rank this dose group had a higher incidence of anomalies which were more predictive of teratogenic potency.

None of the soft tissue anomalies observed in rats or mice was increased in a clear dose-related manner (Tables 7 and 8). However, in fetuses from rats that received 2,000 mg/kg/day there was a statistically significant increase in lateral hydrocephalus and the various summaries of anomalies by rank. In addition, fetuses from this group also appeared to have a higher incidence of hydrocephalus of the third ventricle. In mice, there did not appear to be an increase in any of the observed anomalies.

None of the skeletal anomalies observed in rats or mice were increased in a clear dose-related manner (Tables 9 and 10). However, in fetuses from rats that received 2,000 mg/kg/day of the formulation there was a statistically significant increase in enlarged frontal fontanel, enlarged occipital fontanel, split centra, and anomalies with a rank of 1 to 4. In addition, fetuses from this group appeared to have a higher incidence of incompletely ossified supraoccipital. In mice, there did not appear to be an increase in any of the observed anomalies.

In another study,[5] maneb or zineb was given orally to rats in doses of 2,000 to 4,000 mg/kg on days 11 or 13 of gestation and a high incidence of neural tube closure defects, cleft lip and palate and skeletal defects resulted. The teratogenic potential of ferbam and thiram was also evaluated in rats and mice.[6] Ferbam administered to rats during organogenesis by gavage at doses of 114 mg/kg reduced the maternal weight gain, litter size, and fetal body weight. Mice survived ferbam treatment during gestation at doses of 228 mg/kg without adverse effects on these parameters. Thiram treatment during organogenesis reduced maternal weight gain and fetal body weight in rats given 40 mg/kg or more. Litter size was decreased in rats given thiram at doses of 136 mg/kg or more. Some mice died at thiram doses of 300 mg/kg; however, development was not affected in the survivors. These fungicides were judged to have little teratogenic activity.

Ethylenethiourea (ETU) arises from the degradation of ethylenebisdithiocarbamate fungicides and is a potent teratogen in rats.[7] ETU was administered during organogenesis at doses ranging from 5 to 80 mg/kg/day. ETU at doses of 10 mg/kg and above produced neural tube closure defects, hydrocephalus and other malformations of the brain, kinky tails, and limb defects.

Chemical analysis of the zineb formulation used in the present teratology study indicated that it contained 85.5% zineb and 0.35% ETU (Table 2). Consequently, animals that were dosed with 2,000 mg/kg/day of the formulation received 1,710 mg/kg/day of zineb and 7 mg/kg/day of ETU.

In conclusion, the present study indicates that (a) rats were more sensitive than mice to adverse effects of the formulation; (b) no adverse effects on maternal welfare or embryonic and fetal development were observed in rats that received 200 or 632 mg/kg/day of the formulation or in mice that received 200, 632, or 2,000 mg/kg/day of the formulation; and (c) maternal welfare and embryonic and fetal development was adversely affected in rats that received 2,000 mg/kg/day. These adverse affects on development may have been related to the maternal effects or ETU contamination of the formulation. Therefore, the present study indicates that the zineb formulation was not teratogenic in mice but did produce anomalies in rats at doses that adversely affected maternal welfare.

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